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# SOME RESULT OF QUALITY PARAMETERS OF "GARDI" TRANSDERMAL PATCH

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***Abstract:*** *The aim of this study was to conduct standardization study and determine quality parameters of "Gardi" transdermal patch. We used "Gardi" transdermal patch consists of extract of radix Aconitum Kusnezoffii, extract of Terminalia chebula, extract of Saussurea lappa L., extract of Acorus calamus L. and Musk of Musk deer. We used some biologically active compounds of the transdermal patch were revealed by TLC. And main biologically active compounds content of the transdermal patch were determined by spectrophotometric methods. The moisture, average weight and parameters of microbiology were determined by Mongolian National First Pharmacopoeia methods. The transdermal patch was light brown colour, unusual smell and rectangular shape.*

*We determined aconitine, costunolide and gallic acid in "Gardi" transdermal patch by TLC. The retention time of aconitine was 63.04±0.2 minutes and amount was 0.169±0.009% by HPLC.*

*The polyphenolic compounds by spectrophotometer method using Folin-Chiocalto reagent as 0.567±0.043%. Quality and safety parameters of "Gardi" transdermal patch determined as: moisture 27.28±0.65 %, average weight 3.969±0.196 g, thickness 0.83±0.2 mm, total bacteria 1x103, mould, Escherichia coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus and Enterobacter were not detected in "Gardi" transdermal patch.*

***Keywords:*** *Gardi patch; transdermal patch; standardization; quality parameters; high performance liquid chromatography; aconitine;*

## INTRODUCTION

Gardi-5 is one of the traditional Mongolian herbal medicines consisting of five herbal medicines: radix *Aconitum Kusnezoffii*, fruit *Terminalia chebula*, radix *Saussurea lappa* L., radix *Acorus calamus* L. and *Musk of Musk* deer.

Garidi-5 has been used in traditional Mongolian medicine as an antibacterial and analgesic agent for treatments of various diseases including typhus, diphtheria, joint conditions, neurological and skin disorders [1, 2].

Nowadays, the pharmacological and chemical research of Gardi-5 traditional prescription has been widely researched. The Gardi-5 traditional prescription have been anti-inflammatory and analgesic effects and determined quality control parameters [3, 4].

Based on the results of these studies, there is a need to develop new forms of medicine for treatment in the arthritis. Therefore, selecting the "Gardi-5" prescription was made to simplify the process of technological development and application, and to prepare Gardi transdermal patch from Gardi-5 traditional prescription.

At the same time, it was necessary to

standardize it, and this study was objected to define some quality parameters of the Gardi patch.

## MATERIAL AND METHODS

This study was carried out at Institute of Traditional Medicine and Technology. We used Gardi transdermal patch prepared from the Gardi-5 prescription. Gardi patch is composed extracts of the five herbs including *Terminalia chebula* Retz., *Aconitum Kusnezofii* Reichb., *Acorus calamus* L., *Saussurea lappa* L., and musk of *Moschus moschiferus*.

We used some biologically active compounds of the transdermal patch were revealed by TLC.

**Determine alkaloid:** To 1 g of the patch add 2-3 drop ammonia and 10 ml petroleum ether for 30 minutes, filter and use the filtrate as the test solution. Dissolve aconitine standard substance in ethanol to produce a solution containing 4 mg per ml as the reference solution. Carry out the method for TLC, using silica gel G as the coating substance and a mixture of hexane, ethyl acetate, methanol (6.4:3.6:2) as the mobile phase. After develop and removal of the plate, dry in air. Spray with Dragendorff reagent [6].

**Determine costunolide:** To 0.5 g of the patch add 10 ml ethylacetate for 30 minutes, filter and use the filtrate as the test solution. Dissolve costunolide standard substance in ethanol to produce a solution containing 4 mg per ml as the reference solution. Carry out the method for TLC, using silica gel G as the coating substance and a mixture of toluene, ethyl acetate (9.5;0.5) as the mobile phase. After develop and removal of the plate, dry in air. Spray with a 2% of vanillin and sulfuric acid in ethanol and heat at 100-1050C [4].

**Determine gallic acid:** To 1 g of the patch add 10 ml 70% ethanol, 10 ml ethylacetate for 30 minutes, filter and use the filtrate as the test solution. Dissolve gallic acid standard substance in ethanol to produce a solution containing 4 mg per ml as the reference solution. Carry out the method for TLC, using silica gel G as the coating substance and a mixture of benzene, ethyl acetate, formic acid, acetone (5;5;2;0.5) as the mobile phase. After develop and removal of the plate, dry in air. Spray with a 2% of iron trichloride in ethanol [6].

**Estimation of total polyphenolic compounds:** The amount of total phenolics was determined according to the Folin-Ciocalteu procedure. The Folin-Ciocalteu reagent (diluted 1:10 in water) and aqueous Na2CO3 (10.75%) were successively added to the extract. The mixture was at 30 min, then reading absorbance at 760 nm. Gallic acid was used to establish the calibration curve, and total polyphenolic content was expressed as percentage [7, 8].

**HPLC method:** We used ODS C18 column (4x250 mm, 5μm), a mixture of acetonitrile and tetrahydrofuran (25:15) as the mobile phase A and a 0.1 mol/l. solution of ammonium acetate (add 0.5 ml of acetic acid per 1000 ml) as the mobile phase B, elute in gradient as the following: at 0-48 minutes mobile phase A is 15-26%, mobile phase B is 85-74%, at the 48-49 minutes mobile phase A is 26-35%, mobile phase B is 74-65%, at the 49-58 minutes mobile phase A is 35%, mobile phase is 65%, at the 58-65 minutes mobile phase is 35-15%, mobile phase is 65-85%. As detector a spectrophotometer set at 235 nm. Dissolve a quantity of aconitine in a mixture of isopropanol and chloroform (1:1) to produce a mixture containing 0.1 mg per ml as the reference solution.

Weight accurately 2 g of the patch, add 7 ml of ammonia and 100 ml of a mixture of isopropanol and ethylacetate (1:1) and weight. Ultrasonicate for 30 minutes, then filter. The filtrate evaporate to dryness under reduced pressure below 400C. Dissolve exactly the residue in 15 ml of the mixture of isopropanol

and chloroform (1:1), filter and use as the test solution.

**Physicochemistry parameters:** The moisture, average weight and parameters of microbiology were determined by Mongolian National First Pharmacopoeia methods [5].

## RESULTS

### 1.Result of Thin layer chromatography

Qualitative test of Gardi patch have been done by thin layer chromatography. The medicinal plant species of Aconitum are a rich source of alkaloids and flavanoids, many of which exhibit broad spectrum of activity [9]. The alkaloids benzoylmesaconine, mesaconitine, aconitine, hypaconitine, heteratisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isotisine, hetidine, hetsinone and benzoylheteratisine have been isolated from tuberous roots of genus Aconitum [10]. We detected aconitine alkaloids in Gardi patch by HPLC and TLC.

In *Terminalia chebula,* 33% of the total phytoconstituents are hydrolysable tannins (which may vary from 20-50%) and are responsible for pharmacological activity. These tannins contain phenolic carboxylic acid like gallic acid, ellagic acid, chebulic acid and gallotannins such as 1,6 di-O-galloyl-β-D-glucose, 3,4,6 tri-O-galloyl-β-D-glucose, 2,3,4,6 tetra-O-galloyl-β-D-glucose, 1,2,3,4,6 penta-Ogalloyl-β-D-glucose [11].

And *Saussurea lappa* L. has various terpenes that mainly have antitumor properties and anti-infammatory, such as costunolide, dihydrocostunolide, 12-methoxydihydrocostunolide, dihydrocostus lactone, dehydrocostus lactone, α-hydroxydehydrocostus lactone, β-hydroxydehydrocos [12]. Than we developed gallic acid and costunolide in the Gardi patch.

The figure 1 shows the results of the TLC in Gardi patch.

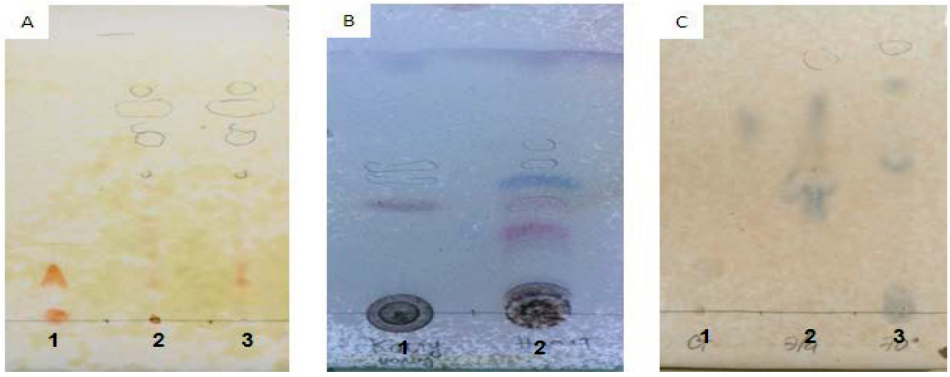


*Figure1. TLC of Gardi patch*

Note: A- Spray with Dragendorff reagent (1– alkaloid, 2–3- extract of Gardi patch), B-5% spray with vanilin-sulfuric acid reagent (1– Costunolide, 2- extract of Gardi patch), C - spray with 3% ferric chloride reagent (1– gallic acid, 2–3- extract of Gardi patch).

We developed alkaloid, costunolide and gallic acid in Gardi patch and Rf values were 0.13, 0.39 and 0.65 respectively.

### 2. Total phenolic contents

The total phenolic contents were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (the stander curve equation: y =110.77 x - 0.0736, r2 = 0.983).

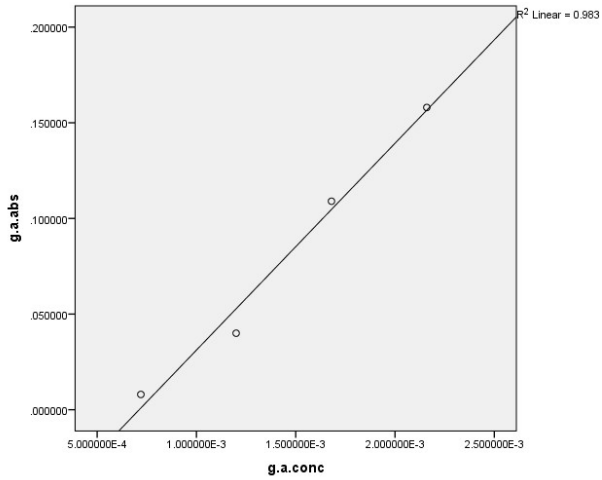


*Figure2. Gallic acid calibration curve*

*Table1. Total phenolic compounds in the extracts of the Gardi patch*

| № | Biological activity substance | Values obtained (%) | Average (%) |
|---|---|---|---|
| 1 | Flavonoids | 0.518 | 0.567±0.043 |
| 2 | | 0.6 | |
| 3 | | 0.585 | |

3. Result of HPLC

The retention time of aconitine was 62.98 minutes.

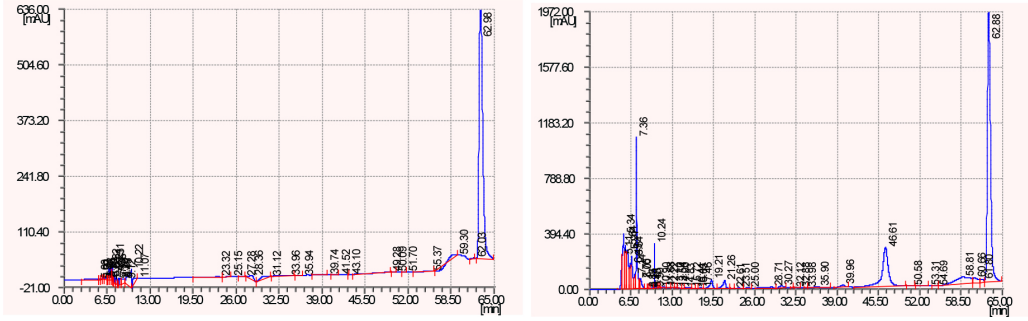


*Figure 3. HPLC chromatograms of aconitine and Gardi patch*

*Table 2. Result of aconitine in Gardi patch by HPLC*

| | Retention time, minutes | Area | Content, % |
|---|---|---|---|
| 1 | 62.88 | 6755892.6 | 0.16 |
| 2 | 63.28 | 6820061.7 | 0.17 |
| 3 | 62.98 | 7125668.8 | 0.178 |
| Average | 63.04±0.2 | | 0.169±0.009 |

The retention time was 63.04±0.2 minutes and amount of aconitine was 0.169±0.009% in Gardi patch.

### 3.Result of physicochemical properties and quality parameters

We determined physicochemical properties and microbiological parameters by Mongolian National First Pharmacopoeia methods.

*Table3. The quality parameters of Gardi patch*

| № | Quality parameters | Methods | Result |
|---|---|---|---|
| 1 | Outer appearance | Organoleptic | Gardi patch was light brown colour, unusual smell and rectangular shape |
| 2 | Qualitative | TLC | Gallic acid, alkaloid, costunolide |
| 3 | Average weight | Weight method | 3.969±0.196 g |
| 4 | Moisture | Weight method | 27.28±0.65 % |
| 5 | Thickness | SATA 91511 | 0.83±0.2 mm |
| 6 | Total phenolic | UV spectrophotometer | 0.567±0.043 % |
| 7 | Content of aconitine | HPLC | 0.169±0.009% |
| 8 | Total bacteria | Microbiological method | $1x10^3$ |
| 9 | Total mould | | Not detected |
| 10 | Escherichia coli | | Not detected |
| 11 | Salmonella | | Not detected |
| 12 | Pseudomonas aeruginosa | | Not detected |
| 13 | Staphylococcus aureus | | Not detected |
| 14 | Enterobacter | | Not detected |

## CONCLUSION

We detected some quality and safety parameters of Gardi patch and such as suitable conditions of TLC to reveal alkaloid, gallic acid and costunolide. The content of aconitine was 0.169±0.009% and the total phenolic content was 7.8±0.67% in the Gardi patch. Quality and safety parameters of “Gardi” transdermal patch was determined as: moisture 27.28±0.65 %, average weight 3.969±0.196 g, thickness 0.83±0.2 mm, total bacteria 1x103, mould, Escherichia coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus and Enterobacter were not detected in “Gardi” transdermal patch. This study is continuing.

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